

The Effect of Tissue Fixation Duration on the Quality of Histopathological Examination Results at Sawerigading Palopo Regional General Hospital

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ABSTRACT

This study aimed to evaluate the effect of fixation duration on the quality of histopathological preparations of prostate tissue using Hematoxylin and Eosin (H&E) staining. A quantitative quasi-experimental design was employed using prostate tissue obtained from biopsy specimens at Sawerigading Palopo Regional General Hospital. Ten tissue samples were subjected to three different fixation durations 6 hours, 24 hours, and 48 hours resulting in 30 observational units. All specimens were fixed in 10% formalin and processed through standard histopathological procedures, including dehydration, clearing, embedding, sectioning, and H&E staining. Histological quality was assessed microscopically based on five parameters: staining quality, nuclear sharpness, cytoplasmic integrity, presence of artifacts, and clarity of histological structure. Data were analyzed using descriptive statistics and inferential tests, including normality testing and nonparametric correlation analysis. The results showed that fixation duration significantly affected histopathological quality. Specimens fixed for 6 hours demonstrated suboptimal morphology characterized by poor nuclear clarity and cytoplasmic integrity, whereas 24 hour fixation produced markedly improved histological features. The highest quality was observed in specimens fixed for 48 hours, showing optimal staining, clear nuclear detail, and minimal artifacts. Statistical analysis revealed a strong positive correlation between fixation duration and histological quality ($p < 0.05$). These findings indicate that fixation duration plays a critical role in determining the quality of prostate histopathological preparations, and a fixation period of 24–48 hours is recommended to obtain optimal diagnostic results.

Keywords: Histopathology, fixation duration; prostate tissue; hematoxylin and Eosin; tissue quality

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INTRODUCTION

Prostate cancer is one of the most common types of cancer in men and has a significant impact on public health. According to data from the Global Cancer Observatory, prostate cancer ranks second as the most common cancer in men globally, with more than 1.4 million new cases recorded in 2020. In Indonesia, the prevalence of prostate cancer is also increasing, especially among men over the age of 60, requiring special attention in terms of diagnosis and clinical management (GLOBACAN, 2025).

Histopathological examination of prostate tissue obtained from biopsy is a standard procedure for detecting malignancy. This process begins with an important stage called fixation, which is a method of preserving tissue that aims to stop the process of autolysis and post-mortem decomposition of cells, as well as maintaining cellular morphological integrity. The most commonly used fixative is 10% formalin, which works by forming cross-links between proteins to preserve the tissue structure as close as possible to in vivo conditions (Chlipala et al., 2020).

The duration of fixation greatly affects the quality of staining results and microscopic interpretation. Fixation that is too quick, such as 6 hours, has the potential to produce tissue that is not fully fixed, causing tissue structure degradation and artifacts. Conversely, fixation that is too long, such as 48 hours, can cause the tissue to become hard and stiff, making cutting difficult and reducing staining quality because the tissue proteins cross-link too much. This shows that an optimal fixation duration is required so that tissue preparations can be analyzed accurately (Digambiro & E dy, 2024).

In practice, varying fixation durations often occur in hospitals, especially in rural areas, including Sawerigading Palopo Regional General Hospital. Limited human resources and heavy workloads in the anatomical pathology unit often result in tissue being fixed for longer than the recommended time, which risks reducing the quality of histopathology results and may lead to incorrect or suboptimal diagnostic interpretations (Bilgin et al., 2023).

In addition to fixation duration, the choice of fixative type can also significantly affect tissue morphology and staining results. There are several other types of fixatives, such as Bouin's solution, formalin-zinc, and alcohol-based fixatives such as ethanol and methanol. The choice of fixative depends on the purpose of the examination, the type of tissue, and the need for further staining. Therefore, inappropriate fixation practices can directly affect the quality and validity of diagnostic results in the context of tissue fixation (Musyarifah & Agus, 2018).

The prostate gland was chosen as the object of study because it has a complex structure with high density, requiring adequate fixation time to allow for even penetration of formalin. The prostate is also highly sensitive to changes in the fixation process, making it an ideal model for evaluating the effect of fixation duration on the quality of histopathological results (Virgiawan et al., 2024). Prostate tissue requires adequate fixation time so that formalin penetration can reach all tissue components evenly. If fixation is too short, the inside of the tissue will undergo autolysis, while if fixation is too long, the protein structure will undergo over-crosslinking, causing tissue hardening and staining disorders. Therefore, prostate tissue fixation must be performed with attention to the appropriate duration, room temperature, and the use of a 10% formalin solution that can maintain stable pH and prevent histological damage (Isaac et al., 2023).

Hematoxylin and Eosin (H&E) staining is a basic technique used in almost all histopathological examinations. However, the quality of the staining results is highly dependent on the fixation stage. Changes in the duration of fixation can cause disturbances in color distribution, uneven staining intensity, and the appearance of artifacts such as silver precipitates, which not only interfere with the morphological appearance but also have the potential to mislead pathological interpretation (Chlipala et al., 2020).

Several previous studies have evaluated the effect of fixation duration on the quality of histopathological staining results. Research by Virgiawan et al. (2024) showed that fixation duration affects the quality of histopathological preparations, where fixation of prostate tissue for 5 hours produced the clearest nuclei and cytoplasm, while 1 hour of fixation produced the worst results. Meanwhile, another study by Likhithaswamy et al. (2022) revealed that storing tissue in formalin for long periods of time has a negative impact on tissue morphology quality. The study results showed changes in specimen color, decreased consistency, difficulty in cutting, and damage to tissue architecture and integrity, including unclear details of the nucleus and cytoplasm.

One crucial aspect that has not been studied in depth in this context is the normality of tissue staining, which reflects the success of the fixation process and is a key indicator of the quality of histological preparations. Inconsistent fixation duration, whether too short or too long, can interfere with the normality of staining. Under-fixation causes tissue proteins to not coagulate completely, resulting in uneven dye absorption, while over-fixation causes excessive protein cross-linking that inhibits dye penetration and produces a blurred and uneven appearance (Likhithaswamy et al., 2022). Therefore, in an effort to improve the accuracy of histopathological diagnosis, assessment of staining normality is a key benchmark to ensure that the fixation procedure has been performed optimally according to the characteristics of the tissue being studied.

Based on this background, where several studies have discussed the effect of fixation duration on tissue quality, there is still a gap in the application of these research results in health care

facilities, especially in areas with limited resources. No study has specifically evaluated the optimal fixation duration at Sawerigading Palopo Regional General Hospital. Therefore, the researchers wanted to further investigate the “Effect of Tissue Storage Fixation Duration on the Quality of Histopathological Examination Results at Sawerigading Palopo Regional General Hospital.”

METHOD

This study is a quantitative study with a quasi-experimental design that aims to determine the effect of fixation duration on the quality of prostate tissue histopathology examination results. The fixation duration variations used were 6 hours, 24 hours, and 48 hours. The study population consisted of prostate tissue obtained from biopsies performed at the Anatomical Pathology Unit of Sawerigading Palopo Regional General Hospital. Accidental sampling was used, with a total of 10 tissue samples, each of which underwent a different fixation treatment, resulting in 30 observation units. The research process includes pre-analytical, analytical, and post-analytical stages. Samples were fixed using 10% formalin, then processed through histopathological stages including dehydration, clearing, embedding, tissue sectioning, and Hematoxylin-Eosin (H&E) staining. The quality of the preparations was assessed microscopically based on five parameters, namely staining quality, cell nucleus sharpness, cytoplasm integrity, the presence of artifacts, and tissue structure differentiation. Data analysis was performed descriptively and inferentially. Univariate analysis was used to describe the characteristics of each fixation duration group, while bivariate analysis used the One Way ANOVA test to determine differences in tissue quality between treatment groups. If the data were not normally distributed, alternative nonparametric tests were used. All analyses were performed at a significance level of 0.05

FINDING AND DISCUSSION

The effect of fixation duration on the quality of histopathological preparations of prostate tissue stained with Hematoxylin & Eosin (H&E) was assessed in three groups of fixation times (6, 24, and 48 hours). Quality was assessed based on five main indicators with a score of 0–2 (poor–good), and a total score ranging from 0–10. The results of the assessment for each duration are presented in Tables 1–3 below:

Table 1. Results of the Effect of Hematoxylin & Eosin Histology Fixation Duration at 6 hours

No	Sample Code	Dyeing Quality (0–2)	Nucleus Sharpness (0–2)	Cytoplasmic Integrity (0–2)	The Presence of Artifacts (0–2)	Histology Details (0–2)	Total Score
1	R1	1	1	0	0	1	3
2	R2	1	1	0	1	1	4
3	R3	1	1	0	1	1	4
4	R4	1	1	0	1	1	4
5	R5	1	1	0	1	1	4
6	R6	1	1	2	1	2	7
7	R7	1	1	1	1	2	6
8	R8	1	1	1	1	2	6
9	R9	2	1	1	1	2	7

10	R10	1	1	1	1	1	5
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Quality score description: Poor (0), Fair (1), Good (2)

Based on Table 1, the results of the histopathological preparation quality assessment of prostate tissue with a fixation duration of 6 hours show that most samples obtained a total score of 4–6, with the lowest score being 3 and the highest being 7. Low scores were generally due to cytoplasmic disintegration and the appearance of tissue artifacts, while staining quality and nucleus sharpness tended to be in the “fair” category. Only a few samples achieved the “good” category in more than two indicators, so that in general the quality of preparations at this fixation duration can be categorized as moderate, with variation in results between samples.

Table 2. Results of the Effect of Hematoxylin & Eosin Histology Fixation Duration at 24 hours

No	Sample Code	Dyeing Quality (0–2)	Nucleus Sharpness (0–2)	Cytoplasmic Integrity (0–2)	The Presence of Artifacts (0–2)	Histology Details (0–2)	Total Score
1	R1	2	2	1	1	1	7
2	R2	2	2	1	1	2	8
3	R3	1	1	1	1	1	5
4	R4	1	1	2	1	1	6
5	R5	1	1	1	1	1	5
6	R6	1	1	2	1	2	7
7	R7	2	2	1	1	2	8
8	R8	2	2	1	1	2	8
9	R9	2	2	2	1	2	9
10	R10	2	1	1	1	2	7

Quality score description: Poor (0), Fair (1), Good (2)

Based on the results of the 24-hour fixation duration assessment of 10 prostate tissue samples, the majority of preparations showed good quality in terms of staining and nuclear sharpness (score 2), with variations in cytoplasmic integrity, the presence of artifacts, and histological detail. The total scores ranged from 5 to 9, with the highest score (9) indicating optimal quality in all indicators, while the lowest score (5) indicated adequate quality in most aspects. In general, 24-hour fixation produced preparations with good and consistent histological quality, although some samples showed a slight decrease in cytoplasmic integrity and histological detail.

Table 3. Results of the Effect of Hematoxylin & Eosin Histology Fixation Duration at 48 hours

No	Sample Code	Dyeing Quality (0–2)	Nucleus Sharpness (0–2)	Cytoplasmic Integrity (0–2)	The Presence of Artifacts (0–2)	Histology Details (0–2)	Total Score
1	R1	2	2	2	1	2	9

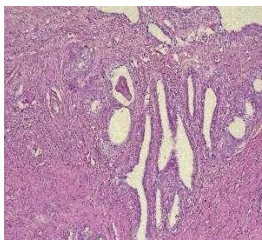
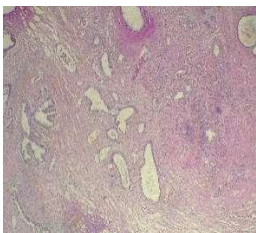
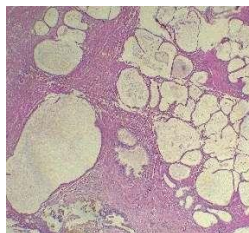
2	R2	2	1	1	1	2	7
3	R3	2	1	1	1	2	7
4	R4	1	2	2	1	2	8
5	R5	2	2	1	1	2	8
6	R6	2	2	2	1	2	9
7	R7	2	2	2	1	2	9
8	R8	2	2	2	1	2	9
9	R9	2	2	2	1	2	9
10	R10	2	2	2	1	2	9

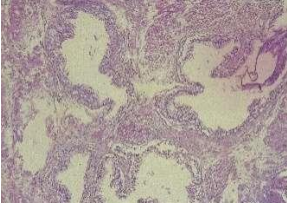
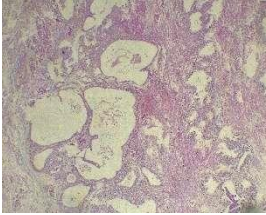
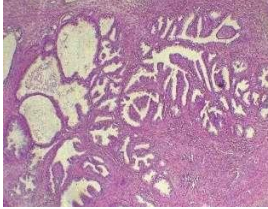
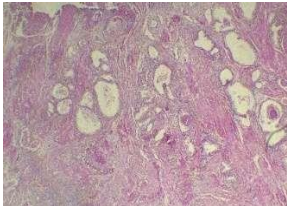
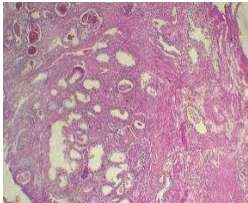
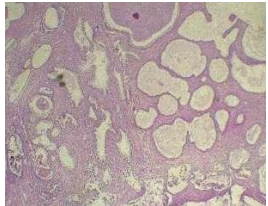
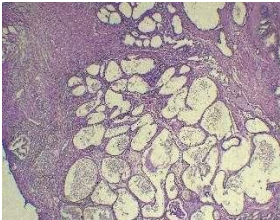
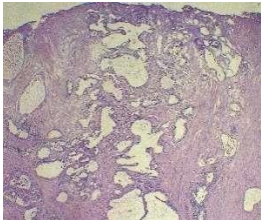
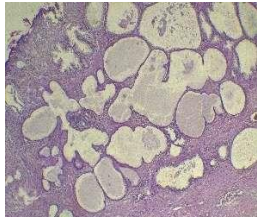
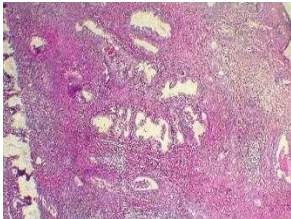
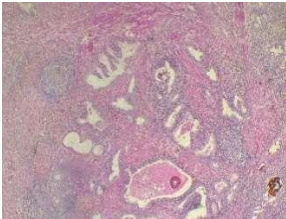
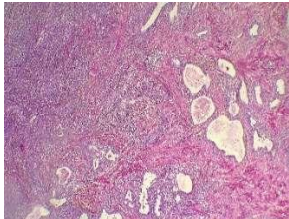
Quality score description: Poor (0), Fair (1), Good (2)

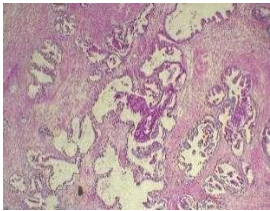
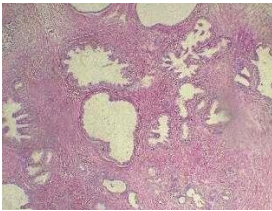
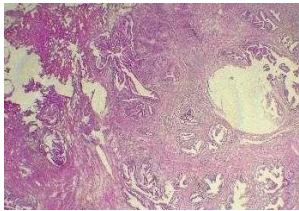
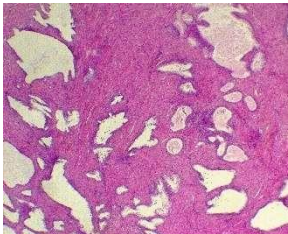
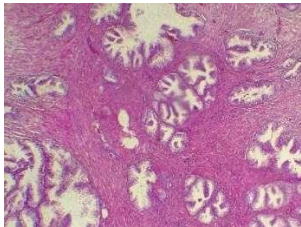
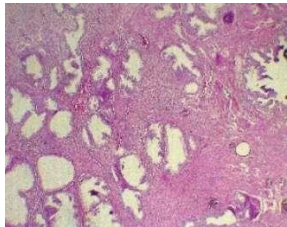
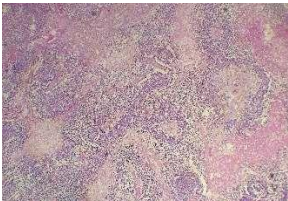
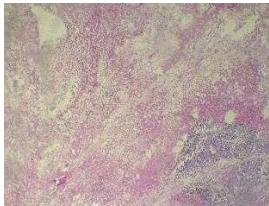
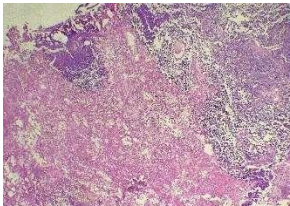
The results of observations over a 48-hour fixation period showed that most prostate tissue preparations had good Hematoxylin & Eosin staining quality, high nuclear sharpness and cytoplasmic integrity, and clear histological details. The total scores ranged from 7 to 9, with the majority of samples scoring 9, indicating optimal tissue morphology quality. However, minor artifacts were still found in all preparations, indicating that prolonged fixation can still cause minor disturbances in histological interpretation.

To clarify the differences in the quality of histopathological preparations at varying fixation durations (6 hours, 24 hours, and 48 hours), the following are visual images of Hematoxylin & Eosin (H&E) staining showing variations in nuclear sharpness, cytoplasmic integrity, the presence of artifacts, and histological details in each group.

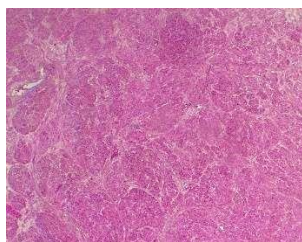
Table 4. Visual Image of the Effect of Fixation Duration

No Sampel	Hematoxylin & Eosin Histology Staining Results		
	6 hours	24 hours	48 hours
1	 The staining quality is unclear, the nucleus is unclear, there is no cytoplasm, poor artifacts, histological details are sufficient.	 Clear staining quality, clear nucleus, unclear cytoplasm, unclear artifacts, sufficient histological detail	 Clear staining, clear nucleus, clear cytoplasm, moderate artifacts, good histological detail

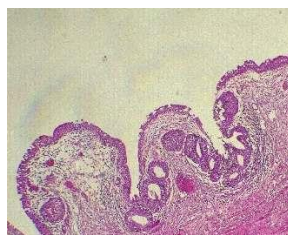
2			
	The staining quality is unclear, the nucleus is unclear, the cytoplasm is absent, there are considerable artifacts, and the histological details are sufficient	Clear staining quality, clear nucleus, unclear cytoplasm, unclear artifacts, good histological detail	Clear staining quality, unclear nucleus, unclear cytoplasm, moderate artifacts, good histological detail
3			
	The staining quality is unclear, the nucleus is unclear, there is no cytoplasm, there are quite a few artifacts, and the histological details are sufficient	The quality of the staining is unclear, the nucleus is unclear, the cytoplasm is unclear, the artifacts are unclear, the histological details are sufficient	Clear staining quality, unclear nucleus, unclear cytoplasm, moderate artifacts, good histological detail
4			
	The staining quality is unclear, the nucleus is unclear, there is no cytoplasm, there are quite a few artifacts, and the histological details are sufficient	Staining quality unclear, nucleus unclear, cytoplasm clear, artifacts unclear, histological details sufficient	The staining quality is unclear, the nucleus is clear, the cytoplasm is clear, there are quite a few artifacts, and the histological details are good
5			
	The staining quality is unclear, the nucleus is	The quality of the staining is unclear, the nucleus is	Clear staining quality, clear nucleus, unclear cytoplasm,

	unclear, there is no cytoplasm, there are quite a few artifacts, and the histological details are sufficient	unclear, the cytoplasm is unclear, the artifacts are unclear, the histological details are sufficient	moderate artifacts, good histological detail
6	 Staining quality unclear, nucleus unclear, cytoplasm clear, artifacts present, histological details clear	 Staining quality unclear, nucleus unclear, cytoplasm clear, artifacts unclear, histological details good	 Clear staining, clear nucleus, clear cytoplasm, moderate artifacts, good histological detail
7	 The staining quality is unclear, the nucleus is unclear, the cytoplasm is unclear, there are considerable artifacts, and the histological details are clear	 Clear staining quality, clear nucleus, unclear cytoplasm, unclear artifacts, good histological detail	 Clear staining quality, clear nucleus, clear cytoplasm, sufficient artifacts, good histological detail
8	 The staining quality is unclear, the nucleus is unclear, the cytoplasm is unclear, there are quite a few artifacts, and the histological details are clear	 Staining quality is clear, nucleus is clear, cytoplasm is unclear, artifacts are unclear, histological details are good	 Clear staining quality, clear nucleus, clear cytoplasm, sufficient artifacts, good histological detail

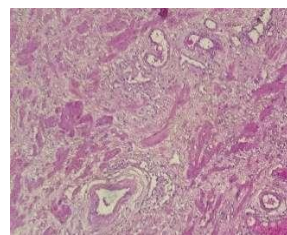
9



Staining quality is clear, nucleus is unclear, cytoplasm is unclear, artifacts are sufficient, histological details are clear

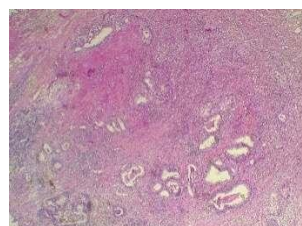


Clear staining quality, clear nucleus, clear cytoplasm, unclear artifacts, good histological detail

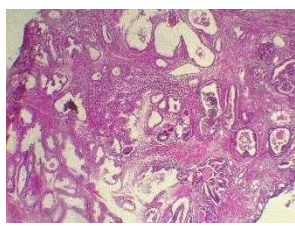


Clear staining quality, clear nucleus, clear cytoplasm, sufficient artifacts, good histological detail

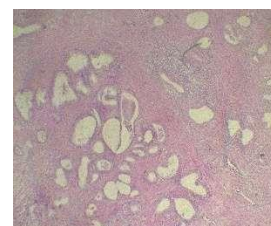
10



The staining quality is unclear, the nucleus is unclear, the cytoplasm is unclear, there are considerable artifacts, and the histological details are unclear



Staining quality is clear, nucleus is unclear, cytoplasm is unclear, artifacts are unclear, histological details are good



Clear staining, clear nucleus, clear cytoplasm, moderate artifacts, good histological detail

Before conducting further statistical analysis, a normality test was first performed to determine whether the research data was normally distributed or not. The normality test is important because the results will determine the type of statistical test used, whether parametric or non-parametric. In this study, the normality test was performed using the Kolmogorov-Smirnov and Shapiro-Wilk methods with a significance level (p-value) of 0.05. The data is said to be normally distributed if the significance value is greater than 0.05, while if it is less than 0.05, the data is not normally distributed. The results of the normality test can be seen in the following:

Table 5. Normality Test

	Shapiro-Wilk		
	Statistic	df	Sig.
Duration of Hematoxylin & Eosin Histological Fixation	0.905	30	0.011

a. Lilliefors Significance Correction

Based on Table 5, with a sample (df) of 30 and the Shapiro-Wilk test, the statistical value was 0.905 and the significance value was 0.011. The significance value was less than 0.05, which means that the data on the duration of Hematocin & Eosin histology fixation was not normally distributed. Therefore, further data analysis uses non-parametric statistical tests because the assumption of normality is not met.

Table 6. Nonparametric Correlations Statistical Test

		Correlations		
			Duration	Result
Spearman's rho	Duration	Correlation Coefficient	1.000	.777**
		Sig. (2-tailed)	.	.000
		N	30	30
	Result	Correlation Coefficient	.777**	1.000
		Sig. (2-tailed)	.000	.
		N	30	30

** . Correlation is significant at the 0.01 level (2-tailed).

Based on the results of the Nonparametric Correlations test in Table 6, it is known that there is a strong and significant relationship between duration and examination results, with a Spearman correlation coefficient value of 0.777 and a significance value (Sig. 2-tailed) of 0.000 ($p < 0.01$). This positive correlation value indicates that the longer the duration within the optimal research limits, the better the examination results obtained. In other words, an increase in duration is directly proportional to an increase in the quality of results, although it is still necessary to pay attention to the duration limits so as not to cause negative effects that can reduce the quality of the examination.

Discussion

Fixation duration plays an important role in determining the quality of prostate tissue histology preparations stained with Hematoxylin & Eosin (H&E). Preparations fixed for 6 hours show relatively low quality, characterized by incomplete cytoplasm, clearly visible tissue artifacts, and suboptimal nucleus clarity. This condition can be explained by the process of underfixation, where formalin penetration is uneven, causing autolysis in the center of the tissue. This phenomenon was also reported by Likhithaswamy et al. (2022), who emphasized that dense tissues such as the prostate require a longer fixation time to ensure that the fixative penetrates to the deepest parts.

Improvements in quality were clearly evident in the 24-hour fixation, with more homogeneous staining, clearer nuclei, and histological details that were beginning to be optimal, although some artifacts were still found. These results are in line with the recommendations of the Royal College of Pathologists of Australasia (RCPA, 2024) and the findings of Meecham et al. (2021), that a fixation duration of 18–24 hours in small to medium-sized tissues is able to maintain morphological integrity, staining contrast, and avoid the effects of overfixation. The research by Digambiro & Edy (2024) also confirmed that this time range is able to maintain a balance between tissue rigidity and dye absorption capacity because the protein cross-linking process occurs stably without causing excessive hardening that can inhibit dye penetration. This cross-linking binds protein molecules within the tissue, making it stable and more resistant to damage.

A fixation duration of 48 hours produced the highest quality preparations in this study, with minimal artifacts and optimal histological detail. Although literature such as Bilgin et al. (2023) warns of the risk of overfixation if exceeding 48 hours, at this duration no significant negative effects have yet emerged. These results reinforce the findings of Virgiawan et al. (2024), who reported that the difference in quality between 24 and 48 hours is relatively small but significant, indicating a plateau point after a certain time, because within that range the tissue has undergone uniform formalin penetration so that morphological stability and stain distribution do not change much even if the fixation time is increased.

Statistical analysis supports this conclusion. Normality tests show that the data are not normally distributed, so a non-parametric approach was used, as recommended by Zulkipli et al. (2024) for ordinal data. The strong positive correlation between fixation time and specimen quality indicates that increasing fixation time within the optimal range is directly proportional to tissue structure stability and staining contrast, as explained by the formaldehyde cross-linking mechanism (Carrillo, 2022). The comparison between groups confirmed the quality order of 48 hours > 24

hours > 6 hours, with a small difference between 24 and 48 hours, indicating the effectiveness of both in meeting diagnostic standards.

Practically, these findings emphasize the importance of implementing a minimum 24-hour fixation SOP in anatomical pathology laboratories, especially in facilities with limited resources. Education of laboratory personnel regarding the risks of underfixation and overfixation is necessary to ensure consistency in specimen quality while maximizing diagnostic outcomes without requiring expensive equipment.

CONCLUSIONS

Based on the results of the study and discussion, it can be concluded that the duration of histological fixation using Hematoxylin & Eosin (H&E) staining has a significant effect on the quality of prostate tissue preparations at Sawerigading Palopo Regional General Hospital, thus proving the research hypothesis. The longer the fixation duration within the optimal range, the better the quality produced, as indicated by an increase in staining parameter scores, nuclear sharpness, cytoplasmic integrity, minimal artifacts, and clear histological details. Fixation for 48 hours provided the best quality across all parameters, followed by 24 hours, which still met diagnostic standards, while 6 hours of fixation produced the lowest quality due to underfixation. Therefore, a fixation duration of 24–48 hours is recommended to ensure optimal histological specimen quality, particularly in high-density tissues such as the prostate.

It is recommended that further research examine the effect of fixation duration on various types of tissue with different sizes and thicknesses, as well as using other types of fixatives besides formalin to compare their effectiveness on the quality of histological preparations. Further research could also integrate quantitative analysis of staining intensity using digital image analysis methods to obtain more precise objective data, as well as evaluate the effects of long-term fixation duration on specimen stability during storage. In addition, studies with a larger number of samples and involving various laboratory facilities will provide a more comprehensive picture and improve the generalization of the results of this study.

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